GLUCOCORTICOID RECEPTOR IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT

Objective: To investigate the relationship among glucocorticoid receptor (GCR) level, immunological classification and clinical efficacy of chemotherapy in acute lymphoblastic leukemia (ALL) children. Methods: The GCR level of venous blood lymphocytes was measured by receptor radioligand binding assay in 50 cases with childhood ALL and 41 normal children. The immunological classification of 32 children with ALL was analyzed by ABC immunoenzymatic method. Results: The GCR number in venous blood lymphocytes of normal children was 4651±1617 binding sites/cell. The normal range (95%) was 1482–7800 binding sites/cell. The GCR level of 50 cases with ALL (6695±5256 binding sites/cell) was significantly higher than that of the normal ones (t=2.50, $P<0.05$). The GCR level of the ALL children with good prognosis was significantly higher than that of bad prognosis (t=4.39, $P<0.001$). The relationship between immunological classification and GCR level of 32 cases with children ALL was as follows: GCR level of T-ALL and B-ALL were significantly lower than AUL, C-ALL and pre-B-ALL; the prognosis of T-ALL and B-ALL was also bad; the GCR level of the group with good prognosis was significantly higher than that with bad prognosis in all immunological types. Conclusion: The GCR level of the peripheral venous blood lymphocytes in children ALL may be an important biochemistry indicator and used to predict prognosis and guide combination chemotherapy. The relationship between GCR and immunological classification can be useful to the expectation of prognosis.

Key words: Leukemia, ALL receptor, Glucocorticoid immunohistochemistry.

Glucocorticoid (GC) has been used in the treatment of childhood acute lymphoblastic leukemia (ALL) for many years. It has proved that the effect of GC is mediated through a glucocorticoid receptor (GCR) of the target cell.[1] Therefore, many experts have concentrated their attention on GCR, but there is no report on the study of GCR in childhood ALL in China. Immunological classification of ALL may differentiate the cell origin and clusters of differentiation (CD) in leukemia cell. It is one of the important indicators to guide combination chemotherapy and to make a prognosis. We used the method of receptor radioligand binding assay to measure the number of GCR in lymphocytes of childhood ALL. We also used ABC immunoenzymatic method to make an immunological classification. This report describes the relationship between the GCR level of lymphocytes of childhood ALL and their efficacy of treatment and prognosis. The GCR level of every immunological type of ALL and its effects for treatment efficacy was also reported here.

MATERIALS AND METHODS

Patients

Fifty in-patients newly diagnosed with ALL were included in this study. The 29 males and 21 females were 1.2 to 12 years old (median 6.6 years). The diagnosis standard was "diagnosis and treatment suggestion of childhood acute leukemia" coming from the Hangzhou meeting in 1986.[2] Patients did not receive chemotherapy before and did not use hormone agents in last two weeks. We used 41 normal children as a comparative group, the 24 males and 17 females were from 1.2 to 10 years old.

Experimental Methods

We used a modified method used by Locabelli, et al.[3] and improved by the study. 4 milliliter venous blood was aspirated from children with ALL at 8 am. The venous blood was added a heparinized tube and lymphocytes were isolated from the freshly obtained samples by the density centrifugation with phosphate

Accepted for publication: June 19, 1999
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buffered solution (PBS). Then, the endogenous hormones were removed. The lymphocyte concentration was made to $5–10\times10^6$/ml. Briefly, the 0.2 ml cell suspensions were incubated (2.5 h at 24°C), the samples were washed three times with 0–4°C PBS. Total and nonspecific binding sites were measured by FJ-2015 liquid scintillometer and the number of binding sites per cell was calculated (unit used is binding sites/cell). Immunological classification was done using Hsu's ABC immunoenzymatic staining, as described previously.[4]

Chemotherapy

All of the patients received the combination chemotherapy protocol including glucocorticoid (COAP, CODP, ect.). After remission induction, L-asparaginase was used as an intense treatment for two weeks. The group with a bad prognosis included non-remission after chemotherapy in 4 weeks, disease deterioration, death and relapse in half a year. The group with a good prognosis included all the other cases.

Statistic Tests

Use *t* test, *u* test, chi-square test and analysis of variance.

RESULTS

GCR Level

The GCR number in venous blood lymphocytes of 41 normal children displayed normal distribution ($u>0.05$). There was no significant difference in the GCR level between boys and girls ($t=0.95$, $P>0.05$). The range of normal reference (95%) was 1482–7800 binding sites/cell. The GCR number of the 50 cases with ALL children did not display normal distribution ($u<0.05$). The GCR level in venous blood lymphocytes with ALL children was significantly higher than that of the normal ones ($t=2.5$, $P<0.05$) (Table 1).

Relationship between GCR Level and Chemotherapy Efficacy and Prognosis

In the 50 cases with ALL, the GCR level of the group with good prognosis (9028±5366 binding sites/cell, n=30) was significantly higher than that of the group with bad prognosis (3195±2411 binding sites/cell, n=20), $t=4.39$, $P<0.001$. Consulting the median of GCR level (5795 binding sites/cell), the ratio of the patients with good prognosis in the group of high receptor numbers was significantly higher than that in the group of low receptor numbers (Table 2).

Table 1. The GCR level of the peripheral blood lymphocytes in normal and children ALL

Groups	Sex	Number of cases	GCR level* ($\bar{x}\pm s$)
Normal	Male	24	4450±1692
	Female	17	4935±1507
	Total	41	4651±1617
ALL	Male	29	6385±5618
	Female	21	7124±4813
	Total	50	6695±5256

* The unit is binding sites/cell.

Table 2. The GCR level of lymphocytes and prognosis in childhood ALL

	Number of cases	Group of good prognosis		x^2	P
		Number of cases	%		
High receptor group	25	23	92	21.33	<0.005
Low receptor group	25	7	28		

GCR Level and Immunological Type

T-ALL

One case had complete remission (CR) and the GCR level was 9287 binding sites/cell. 5 cases had no remission with the GCR level being 4270±4023 binding sites/cell.

Non-T-ALL

B-ALL: one case had CR and the GCR level was 8808 binding sites/cell. Two cases had no CR with the GCR level being 3288±1515 binding sites/cell.

Common-ALL: 14 cases were CR and the GCR level was 8673±4624 binding sites/cell. Three cases had no CR and the GCR level was 2359±1458 binding sites/cell. To compare the GCR level of the CR group and no-CR group in the Common-ALL, the results were $t=2.288$, $P<0.05$.

Pre-B-ALL: Three cases had CR and the GCR level was 13784±13502 binding sites/cell. One case had no CR and the GCR level was 1840 binding sites/cell.

AUL: One case had CR and the GCR level was

14775 binding sites/cell. One case had no CR, the GCR level was 5472 binding sites/cell.

DISCUSSION

Glucocorticoids are extensively used for the clinical. However, there is no report about the normal range of GCR level in venous blood lymphocytes of normal children in China. We measured the GCR level of venous blood lymphocytes in the normal children with a receptor radioligand binding assay. The mean of the GCR number was 4651±1617 binding sites/cell. The normal range (95%) was 1482–7800 binding sites/cell. There was no deference between males and females according to statistical analyses. The study shows that the normal range of lymphocytic GCR level can be used for GCR study about childhood lymphocytic diseases clinically. According to the GCR number of 50 cases with ALL children measured in this study, we found that the GCR number of ALL children was unstable. The range was 515–28970 binding sites/cell, and did not display a normal distribution. The GCR number of ALL children was significantly higher than that of the normal ones. The reason may be that there was a good many blastocytes in the sample of ALL children. Smets, et al.[5] showed that the synthesis of GCR is mainly at G1 phase of the cell cycle and there is a higher content of GCR at S and G2 phases of the cells.

GC has been used in the treatment of ALL children for several decades. Currently, all of the combination chemotherapy proposals treating childhood ALL included GC. However, GC resistance was seen in 10–30% of untreated ALL patients, and is much more frequent in relapsed ALL patients.[6] If an effective way to predict the case's responsible to GC can be found, it would be an objective basis for selecting chemotherapy. It has proved that the effect of GC is mediated through GCR of the target cell.[1] Since 1973, Lippman, et al. first proved that there was GCR at the peripheral venous blood lymphocytes in childhood ALL, many experts in other countries have concentrated their attention on studying GCR. Some experts' study shows that the patients with GCR level less than 4500 binding sites/cell were not sensitive to the treatment of dexamethasone. Furthermore, the patients with low GCR level had a short remission period and high relapse rate. However, there are reports with different results.[7] We measured the GCR number of venous blood lymphocytes in the 50 cases with ALL children. The results were that the median (M) of GCR number was 5795 binding sites/cell. On the demarcation of M (5795 binding sites/cell), the cases with GCR which number more than 5795 binding sites/cell which we named as the high receptor group; the others we named as the low receptor group. 23 cases of the high receptor group were named the group with good prognosis (92%), and seven cases of the low receptor group belong to the group with bad prognosis (only 28%). The result is significant according to the stastical tests. In our sample, 30 cases belong to the good prognosis group and 20 cases belong to the bad one. Contrasting the two groups, we found the GCR number of the group with good prognosis was significantly higher than that with bad prognosis. The results show that the GCR number of lymphocyte may be an important biochemistry indicator and may be used to predict prognosis and guide combination chemotherapy in childhood ALL. For the case with low receptor number, we should adopt an intense chemotherapy proposal and keep the period of maintenance treatment long enough after remission.

Both immunological classification and GCR level in childhood ALL can be used as indexes to predict prognosis, but the relationship between immunological classification and GCR level is not understood yet. Quddus, et al.[8] reported that the GCR number of pre-B-ALL was significantly higher than that of T- and B-ALL and the patients with high receptor number have a higher rate of remission. We studied the relationship between immunological classification and GCR in 32 cases with childhood ALL. The results show that the GCR level of T-ALL and B-ALL were significantly lower than AUL, C-ALL and pre-B-ALL; the prognosis of T-ALL and B-ALL was also bad. The rates of the patients of good prognosis group in T-ALL and B-ALL were 16.7% and 33%, respectively. However, the percentage of the patients of good prognosis group in AUL, C-ALL and pre-B-ALL were 50%, 82.4% and 75%, respectively. The GCR levels of T-ALL and B-ALL were low and their prognosis was also bad. The GCR levels of the group with good prognosis were significantly higher than those with bad prognosis in every immunological classification. The study shows that GCR is consistent with immunological classification when predicting prognosis of ALL. The study of the relationship between GCR and immunological classification is useful to predict prognosis and to guide chemotherapy clinically.

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Rb1 GENE MUTATIONS IN OSTEOSARCOMA

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Genetic alternations, such as mutations caused inactivities of tumor suppressor gene, have been identified in a wide variety of tumors, including osteosarcoma. Osteosarcoma is the most frequent primary malignant bone tumor that occurs in the extremities of young adolescents in most cases. Because of the high frequent occurrence of this type of tumor in hereditary retinoblastoma patients, involvement of the Rb1 gene mutations was suspected in the development of osteosarcoma, and a few reports have shown alternations of the Rb1 gene in osteosarcoma. We studied Rb1 gene mutations in 9 osteosarcoma samples and one cell line (OS 732) to explore the types and mechanism of Rb1 gene mutations in osteosarcoma.

MATERIALS, METHODS AND RESULTS

Tissue samples were obtained from osteosarcom patients diagnosed by clinic and pathobiology from 1994 to 1995. The control samples were normal peripheral blood obtained from normal persons without immediate family or past history of malignant disease. Genomic DNAs from above samples were extracted according to the convention method, and the 27 exons of Rb1 gene were amplified by PCR respectively. The diluted PCR products were denatured and then loaded on 8% nondenatured polyacrylamide gels. Electophoresis' were performed with constant power of 30W for 3 to 6 h at room temperature. Silver staining was used to develop the gels. There were four samples appeared abnormal band shifts compared with control samples. Direct sequencing of the PCR fragments from all the samples were performed. There is a mutation found in one of the samples. The mutation occurred in the position of Rb1 cDNA 1804 which is a C to T single base substitution. The mutation leads the arginine codon CGA changed into stop codon TGA.

DISCUSSION

Recently, the results from osteosarcoma study showed that the abnormal expression of Rb1 gene or alterations of Rb1 protein's function played a very important role in the development of osteosarcoma. There are no "hot spots" of mutation in the Rb1 gene in retinoblastomas as well as in other malignancies. Based on the optional condition of PCR-SSCP, we have found 4 samples had abnormal bandings compared with control samples, and they distributed in four exons. One of these four samples was demonstrated to exite a C→T single base substitution. This mutation results in a codon CGA changed into a stop codon TGA.

Mutations caused prematured stop codon in Rb1 gene is a common reason that leads inactivity of Rb1 protein. The mutation of C→T is usually considered to be due to the higher mutation rate of 5-methylcylosine in CpG. And the characterized flanking seguence around mutation point may be another important factor causing mutation. Previous studies have indicated that almost 50% mutations in Rb1 gene in retinoblastomas involved the CGA coden. Our finding is similar to that of Bunichioro wadayama et al.. According to our result and others, we concluded that exons with CGA code involved should be considered as relative hot spots when detecting mutation in Rb1 gene. We will adjust the experimental conditions for sequencing in order to look for higher mutation detection rate to do the further molecular mechanism study for the osteosarcom.

(Accepted for publication: June 18, 1999)